



Preparation and Properties of Non-Crosslinked and Ionically Crosslinked Chitosan/Agar Blended Hydrogel Films

MOHAMED MAHMOUD NASEF, ESAM A. EL-HEFIAN^{§*},
SARIAH SAALAH and ADUL HAMID YAHAYA[§]

Chemical Engineering Department
Faculty of Chemical and Natural Resources Engineering
Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia

[§]Chemistry Department, Faculty of Science,
University of the Western Mountain, Azzawiyah, Libya
eelhefian@yahoo.com

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Abstract: Hydrogel films of chitosan (Cs) and agar blends of various proportions were prepared using physical solution blending. Some of the obtained films were ionically cross-linked by treatment with calcium chloride solution. The obtained films were characterized by Fourier transform infrared (FTIR) spectroscopy, thermogravimetry analysis (TGA), differential scanning calorimetry (DSC) and universal mechanical tester. The non-crosslinked Cs/agar blended films showed lower water swelling, melting temperature, stiffer and less elastic properties than that of pure Cs films and higher than pure agar films. Crosslinked blended films displayed further reduction in the swelling and melting temperature while maintained more hydrogel stability.

Keywords: Chitosan, Agar, Blending from solution, Ionic crosslinking, Hydrogel films

Introduction

Polysaccharides are a class of natural polymers which have been receiving increased attention in various fields of industrial interest including food technology, pharmaceuticals and waste water treatment¹. Among all, chitosan (Cs) which is a cationic polysaccharide obtained by alkaline deacetylation of chitin, the principal exoskeleton component in crustaceans occupies a special position. This is due to its abundance, versatility and ease of modification together with possession of a combination of unique properties including biodegradability, biocompatibility, non-toxicity, anti-bacterial nature and hydrophilicity^{2,3}. This has promoted the use of Cs in a broad range of applications in medical, pharmaceutical, chemical, agricultural and environmental fields.

Agar, an unbranched polysaccharide, is a hydrophilic colloid extracted from the family of seaweeds (Rhodophyceae) having the structure of 1,4-linked- 3,6 -anhydro- α -L-galactopyranose⁴. One of the most important properties of agar is its ability to form reversible gels even at low concentration simply by cooling its hot aqueous solutions due to the formation of hydrogen bonding⁵. This has given agar a wide range of application as thickening and gelling agent in a variety of industries^{6,7}. However, blending of agar with natural or synthetic polymers to modify its properties has been very scarce in literature. For example, hydrogel blends of agar with polymers such poly(vinylpyrrolidone) (PVP), have been reported⁸.

Blending of Cs with other natural or synthetic polymers has been proposed as convenient and effective means to improve its physical properties to meet the requirements for some practical applications^{9,10}. Studies on natural polymer blended membranes or films such as Cs/cellulose¹¹, chitosan/starch¹², Cs/gelatin¹³ and Cs/collagen¹⁴ have been reported in various occasions. However, reports on blended membranes of Cs/agar have not been reported. The similarity of agar and Cs in their primary structures (Figure 1) suggests that there is a high possibility for the formation of homogeneous blend with improved physical properties. The combination of the properties of the two gel forming polyionic polymers and ion crosslinking with bi-cations such as Ca^{2+} from calcium chloride would produce a hydrogel blend possibly suitable for biomedical applications involving entrapment and encapsulation strategies.

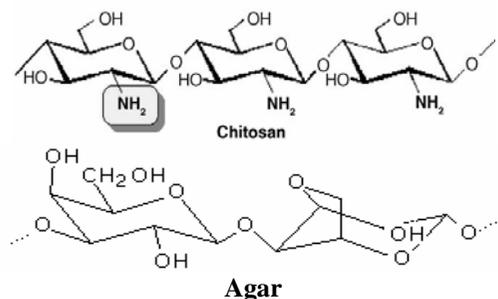


Figure 1. Chemical structures of chitosan and agar

The objective of this study is to report on the preparation of Cs/agar blended films made at different proportions with and without ionic crosslinking, which was performed by calcium chloride solution. FTIR spectral analysis was used to study the variation in the chemical composition of the Cs/agar blends and the formation of crosslinked structure. The obtained blend films of Cs/agar were also investigated to evaluate their swelling behavior. The thermal behavior of the blended films were also investigated using DSC analysis.

Experimental

Shrimp source Cs in a form of white flakes with a degree of deacetylation of 88.1% defined by UV method¹⁵ was obtained from the chitin-chitosan laboratory at the National University of Malaysia (UKM), Malaysia. Agar was purchased from Sigma Chemicals Co. (UK). Acetic acid (glacial 100%, pro analysis) was purchased from Merck (Germany). Calcium chloride anhydrous (granular, $M = 110.99 \text{ g mol}^{-1}$) was supplied by R & M Marketing (Essek, United Kingdom). Ultra pure water (Elga-Prima Corp, UK) with a resistivity higher than $18 \text{ M}\Omega \text{ cm}^{-1}$ was used to prepare all solutions. All chemicals were used without further purification and freshly prepared solutions were always used in all experiments.

Preparations of the solutions

Cs was grounded and dried in an oven until a constant weight was observed. A 10 g L⁻¹ solution of Cs was prepared by dissolving 5 g of chitosan in 500 mL of acetic acid (0.1 M) followed by stirring and heating at 60 °C for 16 h. The solution was filtered before being used to remove the undissolved particles. The agar powder was dissolved in preheated ultra pure water followed by stirring at a temperature of 90 °C until clear solutions were obtained (after about 2 hours). To prepare the Cs/agar blends, Cs and agar solutions were mixed at the prescribed compositions and stirred at 90 °C for 30 min. to obtain homogeneous blended solutions. The pH of the solution was maintained at levels higher than pH~6 to avoid Cs precipitation.

Preparations of the films

The obtained homogeneous Cs/agar solution was cast into a polystyrene petri dish, after removing all trapped air bubbles. The cast solution was then allowed to dry in a vacuum oven for 48 h at 60 °C to form uniform and homogenous film with a thickness of about 14±2 µm. The films were then kept under evacuated dessicator over dried silica gel to remove the traces of residual solvent. A series of blended films were prepared by changing the content of agar in the blend in the range of 5-25 vol% according to the proportions shown in Table 1. Film samples with the same thickness based on the parent pure Cs and agar polymers were also prepared and used as references. The films thicknesses were obtained at various positions of the samples using a digital thickness gauge and the reported data is the average of 10 readings.

Table 1. Composition of agar/chitosan blended films

Films designation	Agar (vol%)	Cs (vol%)
Pure Agar	100	0
Pure Cs	0	100
Cs/Agar (95/5)	5	95
Cs/Agar (90/10)	10	90
Cs/Agar (85/15)	15	85
Cs/Agar (80/20)	20	80
Cs/Agar (75/25)	25	75

Ionic crosslinking of the films

The blended films were ionically crosslinked using the method described by Huang *et al.*¹⁶. The dried Cs/agar films were immersed in a 5 wt% calcium chloride in 70 wt% aqueous ethanol solution. The crosslinking reaction was allowed to continue for 30 min at room temperature under continuous stirring. The crosslinked (CR) films were removed, washed with pure water to remove the residual calcium chloride and then placed under vacuum for drying and storage. Similar crosslinked films of pure CS and pure agar were also obtained.

FTIR spectral analysis

FTIR-ATR spectra of samples in film forms having the same thicknesses of 14 (±2) µm were obtained by a Nicolet spectrometer (Avatar 370 DTGS) equipped with an attenuated total reflectance (ATR) having Ge crystal at 45°. Measurements were performed in a frequency range of 4000-400 cm⁻¹ with a 4 cm⁻¹ resolution and 20 scans.

Swelling test

Swelling test of the films was performed by soaking a dry film of known weight into pure water at room temperature until swelling equilibrium was achieved after 12 h. The equilibrated film was removed and water adhering to its surfaces was plotted with tissue papers before its final weight was recorded. The degree of swelling was calculated using the following equation:

$$\text{Degree of swelling} = \frac{(w_s - w_o)}{w_o} \times 100\%$$

Where w_o and w_s are the weights of the dry and the swollen films, respectively. All the recorded data are average of triplicate readings.

Differential scanning calorimetry (DSC)

The DSC thermograms of pure Cs, agar and their blended films were measured on a Mettler Toledo DSC (822e) under inert atmosphere. Measurements were performed over a temperature range of 50–400 °C at a heating rate of 10 °C min⁻¹ in sealed aluminum pans. The thermal scans (only first heating run) were repeated three times using three weights of the same film sample and the recorded data represent average values.

Mechanical properties

The mechanical properties were measured using a universal mechanical tester (Instron, Model 5566, USA) according to the ASTM D 882-91. Dumbbell-shaped specimens of 50 mm long with a neck of 28 and 4 mm wide were used. The measurements were carried out at 25 °C and 50% relative humidity. The crosshead speed was fixed at 50 mm/min. A minimum of five specimens were tested for each sample and the average was recorded.

Results and Discussion

Chemical structure of the films

FTIR-ATR spectra of the pure Cs and its corresponding crosslinked films are shown in Figure 2. As can be seen in pure Cs spectrum, the absorption peak at 3360 cm⁻¹ is associated with -OH group while the absorption peaks at 1641 and 1549 cm⁻¹ are assigned to the NHCOCH₃ (amide I) and -NH₂ groups (amide II), respectively. The peak size representing -NH₂ group was found to decrease when the film was crosslinked by calcium chloride. This was coupled with a similar decrease in the size of -OH representing peak suggesting a possible involvement of Ca²⁺ with -NH₂ and -OH groups in formation of polyanionic-cationic complexes resembling a stabilized isotropic gel network of less water content. Similar complex formation behavior was observed for Cs when crosslinked by glycerol phosphate disodium by Chenite *et al.*¹⁷.

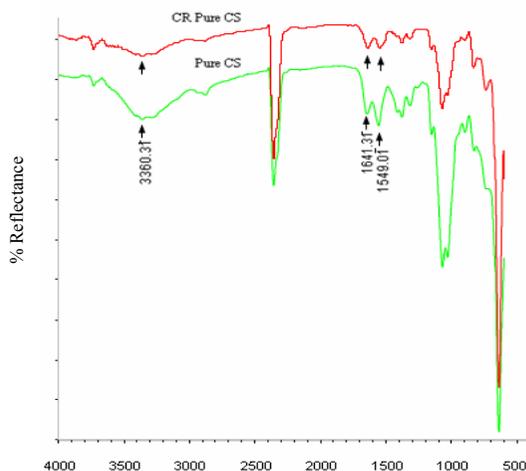


Figure 2. FTIR-ATR spectra of pure chitosan film and its crosslinked counterpart

FTIR-ATR spectra of the pure agar and crosslinked agar films are shown in Figure 3. Both spectra of pure and calcium chloride crosslinked agar showed similar spectra representing the existence of β -D galactopyranose in their molecular structure with some changes can be observed in crosslinked agar spectrum. For instance, the -OH absorption peak at 3393 cm^{-1} was found to decrease indicating a significant water loss caused by crosslinking⁷. The peaks at 1067 and 932 cm^{-1} which were assigned to the 3,6-anhydro-galactose bridges together with the peak at 889 cm^{-1} belonging to the C-H of residual carbon β -galactose also showed a size decreasing tendency. Such features suggest the formation of isotropic gel network of less water content. Contacting calcium chloride with agar most likely induced an immediate interfacial ionic polymerization of the agar through binding of Ca^{2+} available in the solution to the -OH groups of the β -galactose, thus forming a polyanionic-cationic gel. This behavior is going along with that of alginates, polyions natural polymer, when treated with calcium chloride solution¹⁸.

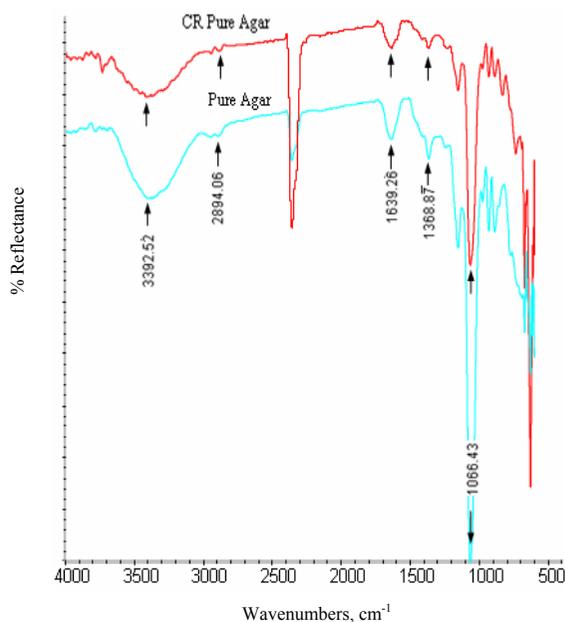


Figure 3. FTIR-ATR spectra of pure agar film and its crosslinked counterpart

Figure 4 shows FTIR-ATR spectra of Cs/agar blend films and their pure polymer counterparts. The spectra of the blended films appear to show a combination of functional groups originated from both parent biopolymers *i.e.* Cs and agar. This includes having -OH and -CH₂OH (methoxyl group) in their backbone. More distinctively, Cs has -NHCOCH₃ groups at 1641 cm^{-1} while agar has a small amount of ester sulfate at 1639 cm^{-1} . Since the peaks of these two groups are too close, the ester sulfate group peak starts to have a masking effect on the peak representing -NHCOCH₃ group, which starts to be influential as agar concentration in the blended films increased beyond 15%.

Figure 5 presents FTIR-ATR spectra of crosslinked Cs/agar blended films together with their non-crosslinked counterparts. The spectra show a similar trend originated from both pure Cs (Figure 2) and pure agar (Figure 3) with and without crosslinking as discussed earlier.

Crosslinking reduced the intensity of $-\text{NH}_2$ peak at around 1554 cm^{-1} . Based on the portrait features, it is confirmed that Cs/agar blended films with various compositional proportions were formed. Moreover, crosslinking has occurred in not only in both pure agar and Cs films but also in their gel blend. The latter is most likely caused by the addition of a Cs to the gelation medium inducing formation of polyanionic–polycationic complexes, which stabilize the ionic gel network and reduce agar water content. Similar behavior was suggested for the addition of chitosan to the gelation medium containing calcium chloride and alginate¹⁸.

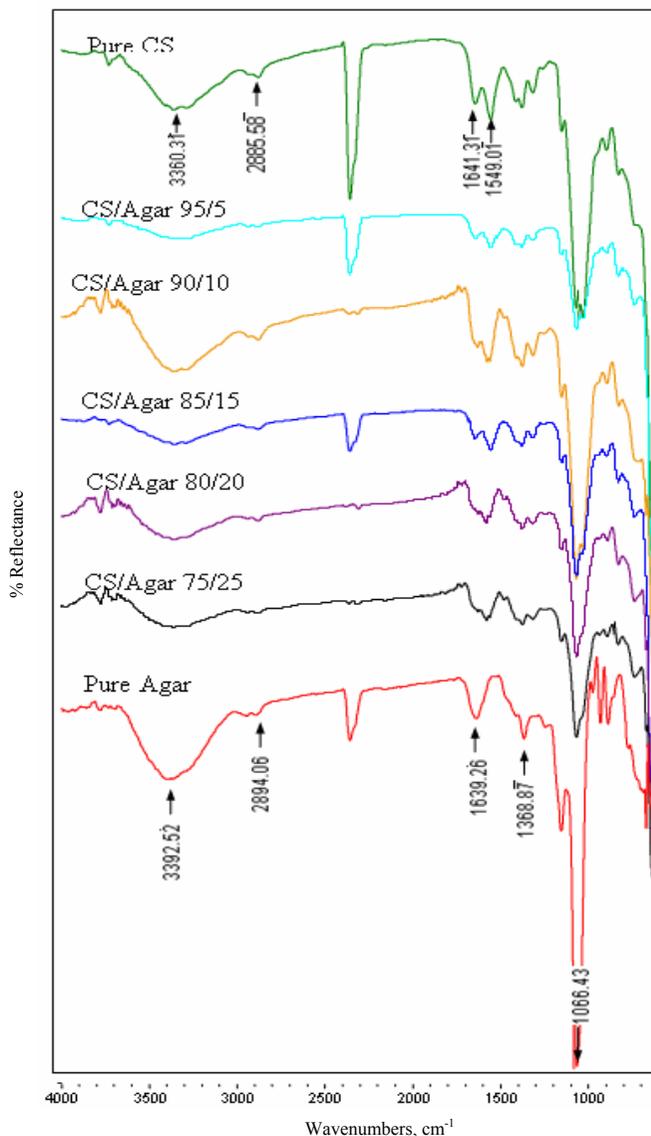


Figure 4. FTIR-ATR spectra of pure agar, pure chitosan and their blended films at various agar proportions

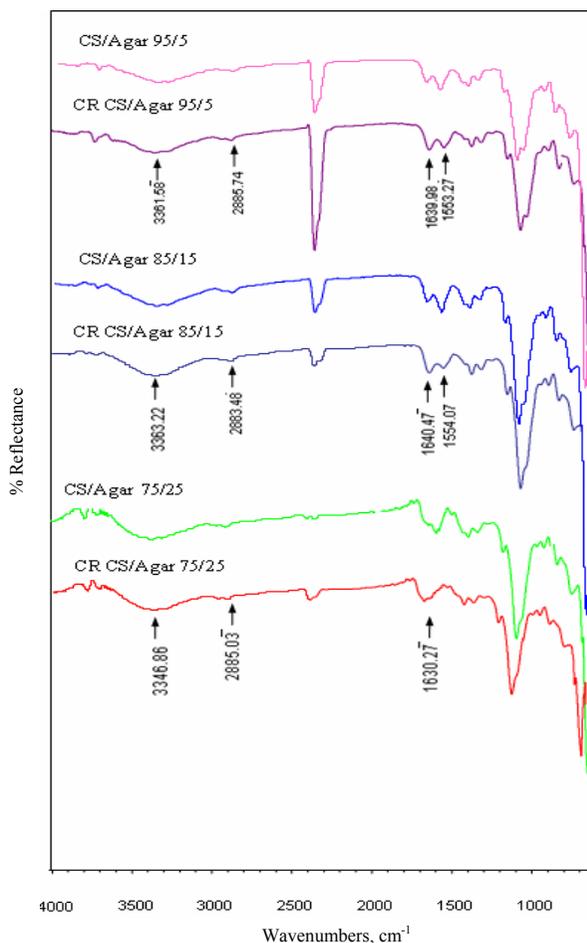


Figure 5. FTIR-ATR spectra of crosslinked (CR) chitosan/agar blended films together with non-crosslinked counterparts.

Water swelling properties

Figure 6 shows the degree of swelling versus the content of agar in the Cs/agar blended films. For instance, the degree of water swelling (at equilibrium) of pure Cs was found to be ten folds (1550%) compared to that of pure agar film (160%). Such great degree of swelling, which confers Cs its hydrogel characteristics were mostly, favored by the protonation and repulsion of chitosan free ammonium groups. Introducing agar to Cs in the blended films was found to considerably reduce the degree of swelling and such reduction is a function of agar content in the blend *i.e.* the increase in the agar content to 5-25% led to a reduction in swelling from 1380 to 1080%.

Crosslinking of the films with calcium chloride was found to reduce the degree of swelling of the pure Cs film by approximately 30% of its original value. The degree of swelling was also found to decrease with the increase in agar content in the crosslinked blended film and it reaches its minimum value (600%) at agar content of 25%. However, the reduction of swelling in the crosslinked Cs/agar blended films was found to be remarkably

higher than that of the corresponding non-crosslinked films having the same agar content. This decreasing trend can be ascribed to the formation of isotropic network structure. These results suggest that both agar blending with Cs and crosslinking with calcium chloride can be used to reduce the swelling of the obtained blended films. The former can lead to a reduction of one third of swelling while the latter further lowered the swelling to almost two third of the original value.

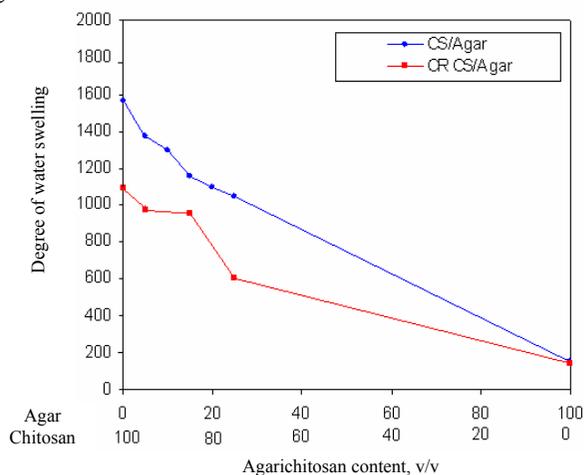


Figure 6. The degree of swelling versus agar/chitosan content in the blended films and their crosslinked counterparts

Thermal properties

Thermal analysis was performed using DSC to study the melting behavior in terms of crystalline melting point, degree of cure, percentage of additives in a polymer, oxidative stability and the degree of crosslinking¹⁹. DSC curves for heating runs (first heating scan) of Cs, agar and Cs/agar blended films are shown in Figure 7. It can be observed that there is an endothermic peak at 112 °C in chitosan film caused by the dissociation process of interchain hydrogen bonding of Cs²⁰. Yang *et al.*²¹ reported an endothermic peak of pure Cs membrane at 125 °C and such thermal behavior was related to the denaturation of the order domains of protein. Such discrepancy is most likely attributed to the difference in source of Cs and degree of deacetylation in both studies. On the other hand, the melting endothermic peak for agar was shown at 83 °C, which is quite close to the melting temperature of similar agar gel reported by Prasad *et al.*²².

The melting endotherm peaks of Cs/agar blended films was found to fall in the range of that of pure Cs and agar as depicted in Figure 8 which shows the variation in melting temperature of CS/agar blend films and their crosslinked counterparts versus the content of agar in the blend. The melting temperature (T_m) of the non-crosslinked blended agar/chitosan hydrogel films was found to have a mixed increase as the agar content increased up to 25%. For instance, the melting peak of the chitosan/agar blended films was shifted from 112 °C for pure chitosan film to 141 °C at 25% agar content in the CS/agar blended films compared to 81 °C for pure agar film. This shift suggests that agar has a synergistic effect on the melting behavior of CS/agar blended films and such effect is most profound at 25% agar content. It can be concluded that addition of agar with contents up to 25% improves the melting behavior of the blended films.

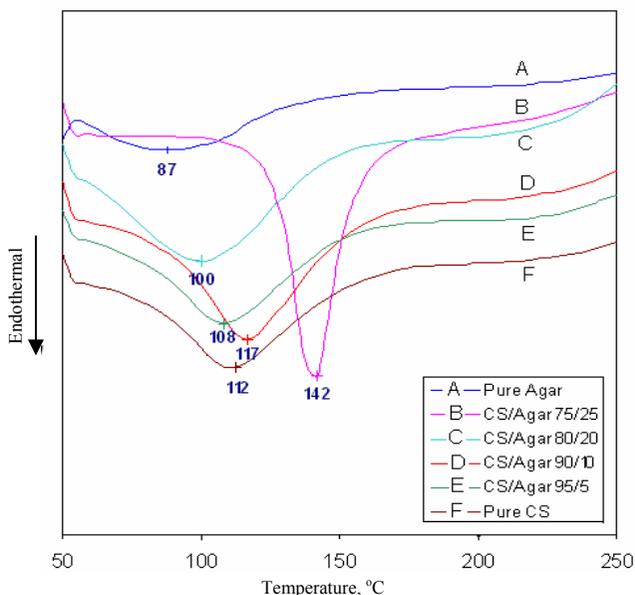


Figure 7. DSC curves of various chitosan/agar blended films

The effect of crosslinking on the melting temperature (T_m) of CS/Agar blended films as a function of agar content is also presented in Figure 8. Crosslinking with CaCl_2 was found to cause a mixed decrease in T_m of CS/agar blended films, which became profound at 15% (T_m dropped to 71 °C) unlike pure Cs film in which T_m dropped 4 °C after crosslinking. In contrast, crosslinking drastically increased T_m of pure agar film from 83 to 138 °C. This suggests that crosslinking improves the melting behavior of the agar due to the formation of network structure unlike crosslinked pure Cs and CS/Agar blended films which causes further loss in swelling and melting temperature.

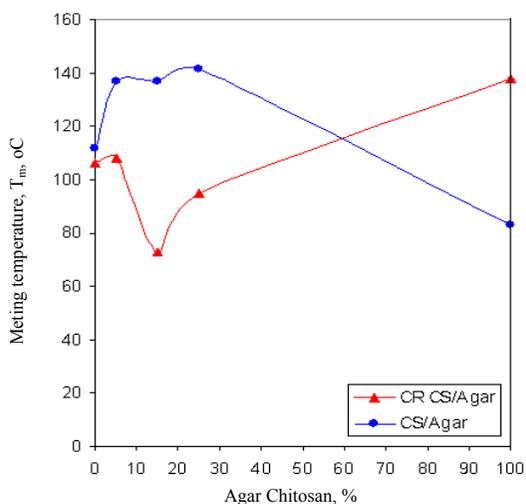


Figure 8. Variation of melting temperature of non-crosslinked CS/Agar blended films and their crosslinked CS/Agar counterparts with the agar content.

Mechanical properties

The mechanical properties in terms of tensile strength and elongation% of both non-crosslinked and ionically crosslinked Cs/agar blended films in correlation with the content of agar are shown in Figures 9 and 10, respectively. As can be seen, the increase in the agar content in the blend film led to gradual reduction in the tensile strength with pure Cs film recording the highest tensile strength value (25 MPa). Similar decreasing trend was observed for elongation% of blended films upon increasing agar content with pure Cs recording the highest elongation% value of 53%. Ionic crosslinking of Cs/agar films with calcium chloride improves the tensile strength and further reduces elongation% with increasing agar content. These results suggest that blending agar with Cs produces films of stiffer and less elastic nature than pure Cs film and such behavior becomes profound upon ionic crosslinking.

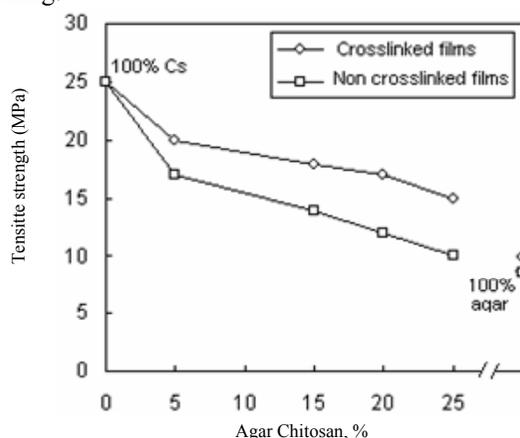


Figure 9. Variation of tensile strength of non-crosslinked CS/Agar blended films and their crosslinked CS/Agar counterparts with the agar content

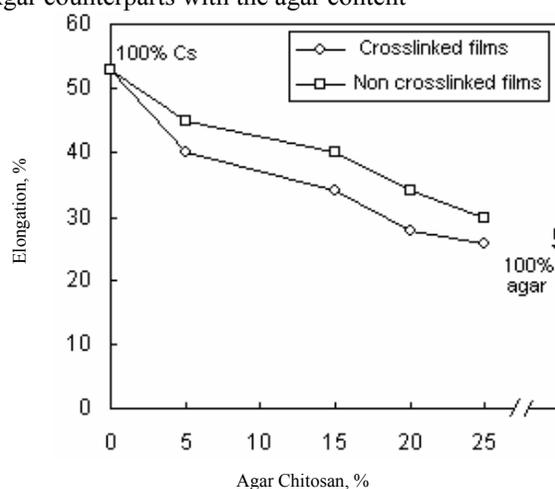


Figure 10. Variation of elongation% of non-crosslinked CS/Agar blended films and their crosslinked CS/Agar counterparts with the agar content

Conclusion

Preparation of films of crosslinked and non-crosslinked pure Cs and agar and their blends at various proportions was investigated. The water swelling of the Cs/agar blended films was found to be reduced by introducing agar and such reduction is a function of agar content in the blend (5-25%). The formation of isotropic network structure in the crosslinked films was found to reduce the degree of water swelling of all films compared to non-crosslinked ones. The T_m of the non-crosslinked hydrogel blended films increased as the agar content increases up to 25% unlike that of the crosslinked films, which tend to have a mixed decreasing trend. The crosslinked Cs films showed a slight decrease in T_m whereas that of pure agar increased drastically. The overall results of this work suggest that blending of Cs with agar produces hydrogel films with improved stability related properties *i.e.* lower swelling and high melting temperatures both of which were further enhanced by ionic crosslinking of the blended films with calcium chloride. Cs/agar blend films were found to have stiffer and less elastic characteristics than pure Cs and such behavior increases by ionic crosslinking while it is a function of the content of agar.

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